A PORTABLE APPARATUS FOR

ESTIMATING STOMATAL APERTURE IN CONIFERS

BY WILLIAM LOPUSHINSKY

PACIFIC NORTHWEST FOREST AND RANGE EXPERIMENT STATION
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Page

PAST WORK

In broad-leaved species, the degree of stomatal opening usually is determined by the porometer method[1] or the silicone-rubber impression method.[2] Unfortunately, these methods are not suitable for conifers because the leaves are small and the stomata sunken and often partially occluded with wax in mature needles. These limitations have led to the development of infiltration methods in which the degree of infiltration resulting from penetration of a solvent or dye solution is used as a general measure of stomatal aperture.[3] Recently, Fry and Walker[4] described a pressure-infiltration method, adapted from techniques used by Froeschel and Chapman,[5] for estimating stomatal opening in conifers. The method involves enclosing a needle in a chamber filled with an ethanol-water mixture and applying pressure until infiltration of the stomata occurs. The pressure required to cause infiltration is considered a measure of stomatal aperture.

Fry and Walker[6] described the use of the method with Douglas-fir and calculated stomatal widths corresponding to a range of infiltration pressures. The present paper describes a compact, portable apparatus designed for use in the field with conifers. Data showing the effect of alcohol concentration, and the relation of infiltration pressures to transpiration rate in ponderosa pine are presented.

1/ Heath, O. V. S. The water relations of stomatal cells and the mechanisms of stomatal movement. Plant Physiol 3: 193-250. 1959.

2/ Zelitch, I. The control and mechanisms of stomatal movement. In Stomata and Water Relations in Plants, pp. 18-42. Conn. Agr. Exp. Sta. Bull. 664. 1963.

3/ Oppenheimer, H. R., and Engelberg, H. Mesure du degre d'ouverture des stomates de coniferes--Methodes anciennes et modernes. (Colloque International de Methodologie de L'Eco-Physiologie Vegetale, Montpellier, 1962) UNESCO Arid Zone Res. 25: 317-323. 1965.

4/ Fry, E., and Walker, R. B. A pressure-infiltration method for estimating stomatal opening in conifers. Ecology 48: 155-157. 1967.

5/ Froeschel, P., and Chapman, P. A new method of measuring the size of the stomatal apertures. Cellule 54: 233-250. 1951.

6/ See footnote 4.

THE INSTRUMENT

DESIGN

Figure 1 shows the needle chamber in an exploded view. The chamber is made from aluminum alloy for light weight. A thick Plexiglas window provides uniform lighting of the interior of the chamber. Use of the O-ring seal permits replacement of windows and cleaning the interior of the chamber. The O-ring and polyethylene washer arrangement on the needle holder permits the needle to be rotated so that all surfaces can be viewed while pressure is being applied to the chamber. This is an important consideration because, with ponderosa pine needles, infiltration often occurs first on the curved surface of the needle and later on the radial surfaces.

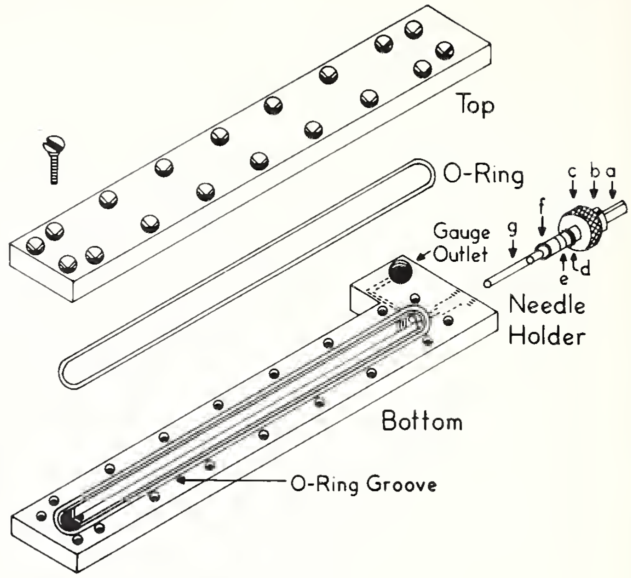

Figure 1.—An exploded view of the needle chamber. Dimensions of bottom are 1/2 by 1-1/2 by 9-5/8 inches. Plexiglas top is 3/8 inch thick. (*a*, flattened stem for rotating needle; *b*, removable retaining ring; *c*, knurled knob; *d*, screw threads; *e*, polyethylene washer; *f*, rubber O-ring; *g*, glass tube for holding needle.)

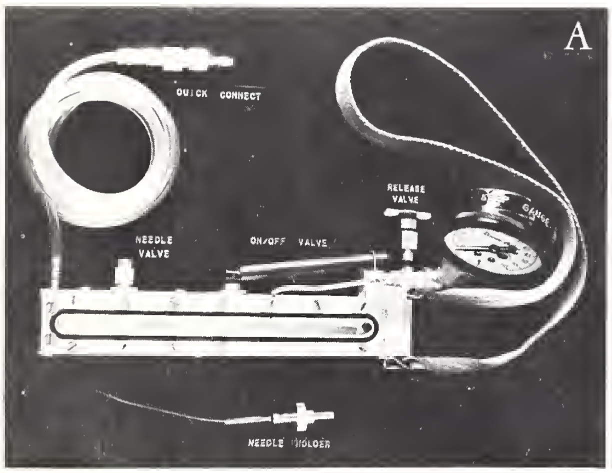

Figure 2.—Assembled needle chamber. *A*, top view; *B*, bottom view.

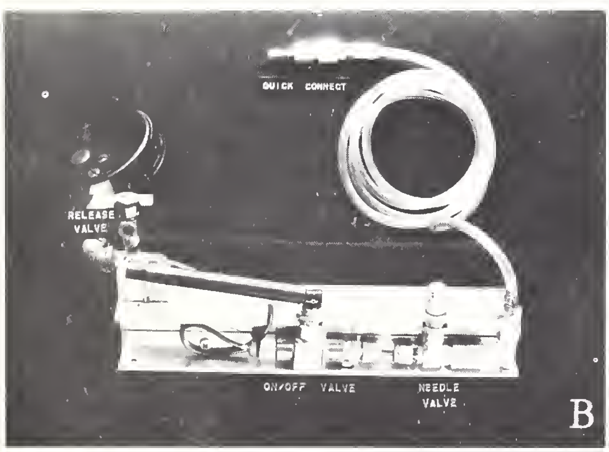

Figure 2 shows the assembled chamber. A lecture bottle of compressed nitrogen, with a miniature gas regulator and quick-connect fitting (see cover photo), is used to apply pressure to the chamber. In actual use, the chamber is supported by a neck strap, preventing alcohol from spilling from the chamber, and the lecture bottle is supported in a holder worn on the hip or placed on the ground. Weight of the chamber is 3.0 lbs. and the gas supply, 6.2 lbs. The lecture bottle permits several hundred determinations from a single filling. Additional items needed for field use are: a plastic wash bottle of ethanol-water solution, hypodermic syringe filled with vasoline, hand lens, and small scissors to cut off needles.

ITS USE

To make a measurement, first insert the base of a needle in the vasoline-filled needle holder which is then screwed into the chamber filled with an ethanol-water mixture. Then depress the off-on lever and close the release valve, allowing

chamber pressure to increase at the rate of about 1.5 pounds per second. A higher rate of pressure increase can be used when measurements indicate that the stomata are partially closed. When a large number of stomata are being infiltrated, release the lever to stop the pressure increase, and record the gauge pressure. Relieve chamber pressure by opening the release valve.

Infiltration of stomata in ponderosa pine needles appears initially as a random scattering of small dark spots. With continued increase of pressure, the number of spots increases and adjacent spots begin to coalesce to form blotches. Observation of infiltration in Douglas-fir needles requires a hand lens. Infiltration appears as a flickering of light reflections[7] or as scattered spots. With both species, pressure increase is stopped when a large number (30 to 50 percent) of stomata are infiltrated. This end point was selected because considerable additional infiltration occurred beyond this point when the pressure increase was stopped and pressure maintained in the chamber.

Temperature changes introduce a potential source of error. The effect of temperature should be slight, however, because the change in surface tension of, for example, a 57-percent alcohol solution is only about 0.0725 dyne per centimeter per degree centigrade. In the temperature range 4.5° to 35° C. (40° to 95° F.), surface tension would be 29.4 and 27.2 dynes per centimeter, respectively, equivalent to alcohol concentrations of 51.5 and 63.5 percent.

EFFECT OF ALCOHOL CONCENTRATION

Mature, current-year needles were cut from lateral twigs of 3-year-old greenhouse-grown Douglas-fir seedlings and allowed to dry under laboratory conditions to gradually close the stomata. At intervals during the drying period, samples of needles were placed on a damp filter paper in a petri dish to prevent further drying, and pressures required to infiltrate needles with 48-percent, 67-percent, and 95-percent ethanol-water mixtures determined.

At the beginning of the drying period when the needles were relatively turgid and the stomata open, the lowest infiltration values were obtained with the 95-percent concentration, followed by the 67-percent and 48-percent concentrations in that order (fig. 3). Values obtained with the 48-percent and 67-percent solutions increased during the first hour of drying, indicating that the stomata had begun to close; however, the values obtained with the 95-percent concentration remained constant. Infiltration pressures increased with further drying for all three concentrations, particularly with the 95-percent ethanol which showed a large increase in infiltration pressure during the second hour. Pressure was not increased beyond 45 p.s.i., and values were recorded as 45+ if infiltration had not occurred up to that pressure. Values of 45+ were

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[7] See footnote 4.

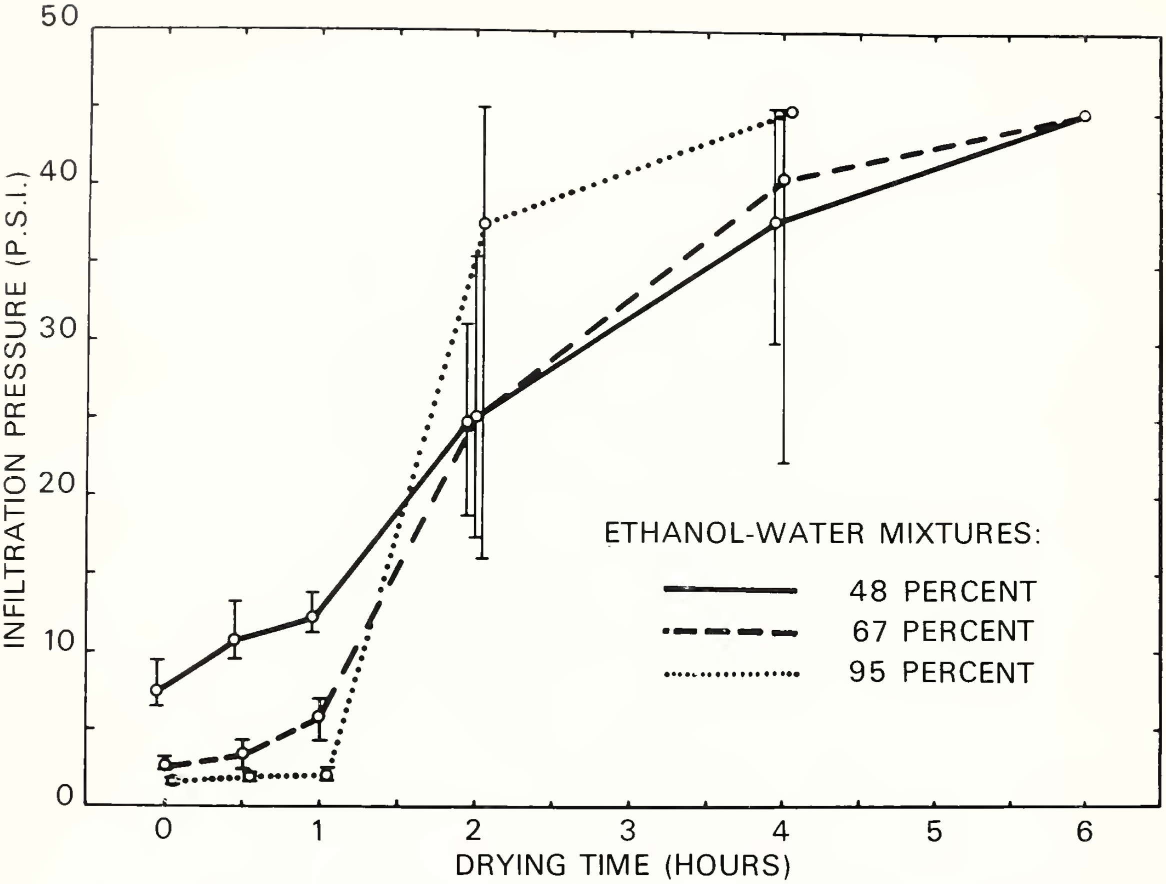

Figure 3.—Effect of alcohol concentration on infiltration pressure in Douglas-fir needles. Each point is an average of six determinations. Vertical bar indicates range of values comprising the mean.

consistently obtained with 95-percent ethanol after 4 hours of drying and with the 48-percent and 67-percent concentrations after 6 hours.

These results indicate that 95-percent ethanol is not a desirable infiltration fluid for Douglas-fir because it does not respond to a small decrease in size of open stomata and has a relatively narrow range of sensitivity in the midrange of closure. The variation between individual values increased with all concentrations as the stomata closed but was usually greatest with 95-percent ethanol. After repeated tests with various concentrations and both greenhouse- and field-grown trees of Douglas-fir, ponderosa pine, and lodgepole pine, a 57-percent solution (60 ml. of 95-percent ethanol diluted to 100 ml.) was selected for routine use. This concentration provides a combination of relatively low infiltration pressure with open stomata, sensitive indication of a slight decrease in size of open stomata, and satisfactory response over the entire range of closure.

RELATION TO TRANSPIRATION

Fry and Walker[8/] calibrated infiltration pressure in terms of calculated stomatal aperture for Douglas-fir and found that the usual range of infiltration pressures of 3 to 45 p. s. i. corresponded to a range of stomatal widths of 3. 5 to 0. 2 μ. Frequently, measurements of stomatal aperture are made to aid in interpretation of transpiration behavior. In the present study, the relationship between transpiration rate and infiltration pressure was determined for ponderosa pine.

A lateral shoot was cut from each of 11 open-grown pines along Swauk Creek near Swauk Pass in central Washington. Growth of the current year was cut off, and the remainder of each shoot (growth of previous year) was allowed to absorb water overnight. The next morning, the base of each shoot was sealed through the lid of a plastic container filled with water to form a potometer. Shoots were then exposed to light for 3 hours in a growth chamber to open the stomata.

Growth chamber conditions were maintained at: air temperature, 21° C.; relative humidity, 59 percent; and light intensity, 3,800 foot-candles (0. 23 g. cal.). After preconditioning in light, a sample of eight needles was removed from each shoot to obtain infiltration values for fully open stomata. The potometers were then weighed at 10-minute intervals to determine transpiration rates. One hour after the start of the weighings, the shoots were moved from contact with water, and a sample of needles was taken from each shoot twice during the ensuing transpiration decline.

Infiltration pressure increased as transpiration decreased, a large decrease in transpiration rate being associated with a small increase in infiltration pressure (fig. 4). There was no effect of needle position within twigs. The average infiltration pressure for fully open stomata was 8. 3 p. s. i.; at 50 percent of maximum transpiration, about 15 p. s. i.; and at 10 percent of maximum, about 40 p. s. i. The data indicate that in ponderosa pine, the stomata can be considered fully open at an infiltration pressure of 10 p. s. i. or less and essentially closed when values exceed 40 p. s. i.

DISCUSSION

The pressure infiltration technique provides a quick and simple method of estimating stomatal aperture in conifer needles in the field. A single determination requires less than 30 seconds, and five to 10 needles usually provide an adequate sample.

Alcohol-water mixtures of 50 to 60 percent appear to be suitable for many species of conifers. Lower alcohol concentrations result in higher values with open stomata, which is not desirable, and higher concentrations, particularly

[8/] See footnote 4.

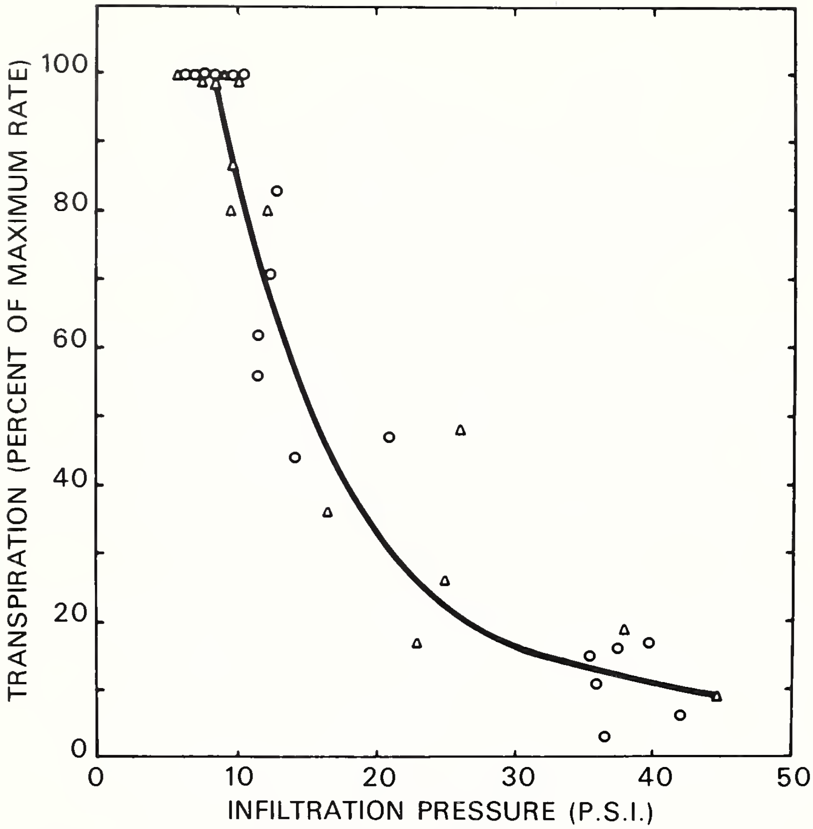

Figure 4.—Relation of transpiration rate to infiltration pressure in field-grown ponderosa pine. Measurements made with a 57-percent ethanol-water mixture. Needles were taken from terminal(○) and midportion(Δ) of growth of the previous year. Each point is an average infiltration pressure for eight individual needles.

95 percent, seem to operate in a rather narrow zone in the midrange of closure. Also, with partially closed stomata, the variability of individual values about the mean increases as alcohol concentration increases. This increased variability probably results because the lower surface tension of the higher concentrations permits entry into a larger range of openings than occurs with solutions of lower alcohol content, and this makes determination of the end point more difficult. The excellent reproducibility of measurements made with open stomata suggests that most of the stomata are open to a uniform width which results in a precise end point. As the stomata begin to close, however, infiltration occurs more gradually over a wider range of pressure, making precise identification of the end point more difficult. This suggests that closure does not proceed uniformly in all of the stomata so that various degrees of closure exist. The completely closed state again represents a condition of relatively uniform narrow aperture resulting in no or very slight infiltration in the range of pressure used.

In addition, infiltration in a long-needled species such as ponderosa pine does not always occur uniformly along the entire length of the needle. In young,

current-year needles of ponderosa pine with open stomata, infiltration often occurs first at a relatively low pressure in the terminal one-third of the needle and later at a slightly higher pressure in the rest of the needle, whereas infiltration in 1-year-old and 2-year-old needles occurs simultaneously over the entire length of the needle. Tip infiltration was observed occasionally in needles of all ages when partially desiccated needles were tested. These observations suggest that the stomata in older tip portions of needles are open to a wider degree than are stomata in younger basal portions. In most instances, 1-year-old (last year's) needles probably are the best choice for testing because they are always present and fully matured, and their stomata probably represent an average in sensitivity between the youngest and oldest needles.

SUMMARY

The design and use of a compact, portable apparatus for estimating stomatal aperture in conifer needles by a pressure-infiltration method is described. Results of a test with Douglas-fir and several ethanol-water mixtures showed that a 50- to 60-percent ethanol-in-water solution was the best infiltration fluid. A transpiration test with ponderosa pine and a 57-percent alcohol solution showed that the stomata can be considered fully open at an infiltration pressure of 10 p. s. i. or less, and essentially closed at 40 p. s. i. Determination of degree of opening was more precise with open and closed stomata than with partially closed stomata, presumably because aperture widths are more uniform in the open and closed conditions. Infiltration in young pine needles often occurred first in the terminal portion of the needle and later in the rest of the needle, suggesting that the stomata in older portions of needles were open to a wider degree than stomata in younger portions.

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